

We claim:

1. A method for producing transgenic animals whose somatic/germ cells contain one or more transgenes, wherein expression of the transgenes result in alteration of the sex ratio of the offspring of said animals, the method comprising the steps of:

- a. preparing a transgene including in operable association (a) at least one expression regulatory sequence (promoter) functional in a post-meiotic spermatogenesis-specific way; (b) a DNA sequence encoding a toxic gene whose expression can interfere with sperm's ability to undergo fertilization; (c) an optional DNA sequence encoding a selectable marker such as neomycin-, hygromycin- or puromycin-resistance gene, Hprt selection cassette, and diphtheria toxin gene; (d) an optional loxP site-flanked intervening DNA sequence inserted between the post-meiotic promoter and the toxin gene, and said intervening sequence can prevent the transcription of the toxin transgene unless it is removed by Cre recombinase; (e) an optional cellular localization signal sequence that restricts the ability of the mRNA and protein from the said transgenes to randomly diffuse among the inter-connected haploid spermatids; and (f) two optional flanking DNA sequences allowing said transgene to be inserted onto specific loci of the sex chromosome (the X or Y chromosome) by homologous recombination method;
- b. creating transgenic animals using the said transgene so that the transgene is inserted onto one of the two sex chromosomes; and
- c. mating the males of the said transgenic animals with animals containing Cre recombinase activity to activate the said transgene and identifying at least one transgenic animal with desirable reproduction feature, specifically, alteration of offspring's sex ratio.

2. The method according to claim 1 wherein said animals include all mammals and non-mammal organisms using X and Y chromosomes to determine sex, and unisexual flower plants.

3. The method according to claim 1, wherein said transgene is selected from the group consisting of Herpes Simplex Virus thymidine kinase gene (HSV-tk), its mutated or truncated genes and any other toxic genes with characters of a) its expression can interfering with sperm's ability to undergo fertilization; and b) its mRNA/protein products act in a no-random diffusion fashion among the inter-connected spermatids.
4. The method according to claim 1 wherein the offspring's desirable sex percentage of said transgenic animals is from 50% to 100%.
5. The method according to claim 1 wherein said post-meiotic spermatogenesis-specific promoter is selected from the group consisting of promoters from HSV-TK gene, protamine family genes, kit, angiotensin converting enzyme (Ace) gene, CaM3 gene, TP-1 gene, TP-2 gene, cytochrome cs gene, PSK-C3 gene, H2B gene, Mea gene, delta-actin gene, proacrosin gene, Idh gene, M-alpha-3, 7 tubulin gene, hsp70.1 gene, Wnt.gene and zinc finger Y gene or any promoter which can trigger post-meiotic expression of said transgenes.
6. The method according to claim 1 wherein said the DNA sequence for X-chromosome specific targeting is Hprt locus or other X-linked sequences whose disruption will not cause abnormal phenotype in transgenic animals.
7. The method according to claim 1 wherein said DNA sequence for Y-chromosome specific targeting is Tspy pseudogene or any Y-linked sequences whose disruption will not cause abnormal phenotype in transgenic animals.
8. The method according to claim 1 wherein the post-meiotic spermatogenesis-specific promoter is replaced with an embryonically-expressed promoter, and the transgene that disrupts sperm's function is replaced by a transgene that interferes with embryonic development or viability, the method further comprising the steps of inserting such transgene onto one of the two sex chromosomes prevents embryos with one particular sex chromosome from developing into individuals, preparing a transgene which comprises in operable association of (1)

at least one expression regulatory sequence (promoter) which expresses in early stage embryos but not during spermatogenesis (for XY organisms) or oogenesis (for ZW organisms); (2) a DNA sequence encoding a toxic gene (such as diphtheria toxin gene and ricin gene) whose expression can kill the embryo or block the normal development of embryos; (3) a loxP site-flanked intervening DNA sequences inserted between the promoter and the toxin gene, and the said intervening DNA sequences can prevent the toxin gene transcription unless it is removed by Cre recombinase; (4) an optional DNA sequence encoding a selectable marker such as neomycin-, hygromycin- or puromycin-resistance gene, Hprt selection cassette, and diphtheria toxin gene; (5) two optional flanking DNA fragments allowing said transgene to be inserted onto specific loci of the sex chromosome by homologous recombination method, creating transgenic animals, and breeding of the transgenic animals with animals that contain a Cre recombinase transgene driven by spermatogenesis- (for XY organisms) or oogenesis- (for ZW organisms) specific promoters.

9. The method according to claim 1 wherein the step of creating transgenic animals includes the steps of targeting the transgene onto the sex chromosome of ES cells using the standard homologous recombination (gene-targeting) method, microinjecting the positive ES cells into recipient embryos to make chimeric mice, and breeding the chimeric mice to pass the ES-derived germ cells to the next generation to obtain the desired transgenic animals.

10. The method according to claim 1 wherein the step of creating transgenic animals includes the steps of targeting the transgene onto the sex chromosome of suitable nuclear donor cells by using the standard homologous recombination (gene-targeting) method, transferring the nuclei of the donor cells into enucleated oocytes by cell fusion or nuclear transfer, and activating the reconstituted oocytes and transfer them into pseudopregnant foster mothers to allow the embryos to develop into individuals with the transgene inserted onto the desired chromosome.

11. The method according to claim 1 wherein the step of creating transgenic animals includes the steps of co-transfecting the HSV-tk transgene with a selection

marker gene into ES or nuclear donor cells by transfection, picking and growing neomycin-resistant clones, examining the transgene integration site by florescence in situ hybridization (FISH), expanding the clones with the transgene inserted onto the desired sex chromosome, and injecting the ES cells into embryos to make  
5 chimeric animals.

12. The method according to claim 1 wherein the step of creating transgenic animals includes the steps of creating transgenic animals from techniques selected from the group consisting of: pronuclear microinjection, retroviral vector transfection,  
10 lipofection, and sperm incubation, and examining the transgene integration site by FISH for each transgenic founder to search for individuals with the transgene inserted onto the desired sex chromosome.